

Extracellular Enzyme Activities of the Monokaryotic Strains Generated from Basidiospores of Shiitake Mushroom

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To obtain basic information on the biochemical property of basidiospores of shiitake mushroom (*Lentinula edodes*), the ability of producing extracellular enzyme was assessed using a chromogenic plate-based assay. For the aim, amylase, avicelase, β -glucosidase, CM-cellulase, pectinase, proteinase, and xylanase were tested against monokaryotic strains generated from forty basidiospores of two different parental dikaryotic strains of shiitake mushroom, Sanjo-101Ho and Sanjo-108Ho. These two parental strains showed different degree of extracellular enzyme activity. No identical patterns of the degree of enzyme activity were observed between monokaryotic strains and parental strains of the two shiitake cultivars. The degree of extracellular enzyme activity also varied among monokaryotic strains of the two shiitake cultivars. Our results showed that dikaryotic parental strains of shiitake mushroom produce monokaryotic basidiospores having very diverse biochemical properties.

KEYWORDS : Basidiospores, Chromogenic media, Extracellular enzyme activity, Shiitake mushroom

Shiitake (*Lentinula edodes*) is an edible and medicinal mushroom belonging to *Tricholoma* genus of Basidiomycota. It grows on broad-leaf trees including *Quercus* spp, *Castanea crenata*, and *Carpinus laxiflor* during spring, summer, and autumn. In the Orient this mushroom has for thousands of years been credited with almost miraculous healing power. It's effect on curing the flu and the common cold, inhibition of the growth of tumors, contribution to longevity, lowering of cholesterol level, and protecting against heart disease has been reported (Hadelar, 1995; Wasser and Weis, 1999). Due to its unique taste and flavor, shiitake has been used in a traditional ingredient of the rich culinary art of the Far East. It has been grown commercially in China, Japan, Korea and Taiwan. Today, the market is rapidly expanding in Asia as well as in North America and Europe. Consequently, it has become the second largest commercially produced mushroom (Hadelar, 1995).

In addition, because of its ability of degrading wood component such as lignin which is hard to break down in nature, shiitake mycelial culture has also been studied as a good source of bioremediation organisms that help clearing up recalcitrant contaminants and as a useful tool of biological agents for bio-pulping in pulp and paper industry (Couto and Herrer, 2006; Okeke *et al.*, 1993). For the better use of shiitake both in mushroom production industry and environmental industry, development of new strains that have improved properties of industrial application has been pursued.

The fruit body of shiitake mushroom is dikaryotic and produces basidiospores that are monokaryotic. Information on the property of basidiospores is very useful for the breeding of more valuable shiitake strain because fruit body forming strain is generated by the cross of monokaryotic strains. So far, it has not been much known about that how much the properties of parental strain appear in the progeny of basidiospores.

In this study, we examined the activity of several extracellular enzymes in monokaryotic strains that are generated from basidiospores of parental shiitake strains to get a basic idea on the use of biochemical properties in the evaluation of basidiospores.

Lentinula edodes strains, Sanjo-108Ho and Sanjo-101Ho (from Forest Mushroom Research Center, Yeosu, Korea), were cultured on PDA at 25°C. Basidiospores were collected from fruiting body of the two strains that had been formed on inoculated oak logs. Monokaryotic strains were generated by germinating the collected basidiospores on PDA at 25°C for 7 days. The identity of monokaryotic stage was confirmed by observing the absence of clamp connection in the mycelium under a light microscope. Each of twenty strains were randomly picked up from the identified monokaryotic basidiospore strains of Sanjo-108Ho and Sanjo-101Ho, named as S108-01 to S108-20 and S101-01 to S101-20 (Table 1 and 2), respectively, and used for extracellular enzyme test.

Extracellular enzyme activities of amylase, avicelase, β -glucosidase, CM-cellulase, pectinase, proteinase, and xylanase were measured on chromogenic media (Hankin and Anagnostakis, 1977; Lee and Lee, 1997; Teacher and Wood,

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Table 1. Extracellular enzyme activities shown by monokaryotic basidiospore strains (S108-01 to S108-20) and its parental strain Sanjo-108Ho

Strains	Enzymes						
	β -Glu	Avi	CMC	Amy	Pec	Xyl	Pro
Sanjo-108Ho	S	W	W	W	S	W	M
S108-01	S	S	S	S	S	S	M
S108-02	M	W	W	S	W	S	M
S108-03	S	M	M	S	M	S	M
S108-04	S	M	W	S	M	W	W
S108-05	S	M	M	S	M	M	M
S108-06	S	M	S	W	W	W	M
S108-07	S	W	M	W	M	S	M
S108-08	S	S	S	W	W	W	M
S108-09	S	W	W	M	M	S	W
S108-10	S	M	M	W	W	W	W
S108-11	M	W	W	S	M	W	M
S108-12	S	W	S	W	W	W	W
S108-13	S	W	M	M	W	W	W
S108-14	S	S	S	W	W	W	W
S108-15	S	M	S	W	W	W	W
S108-16	S	M	W	W	W	W	M
S108-17	S	W	S	W	W	W	W
S108-18	S	W	M	S	M	S	W
S108-19	S	S	S	M	W	W	W
S108-20	S	M	S	W	W	W	W

S, Strong activity; M, Moderate activity; W, Weak activity. β -Glu, β -glucosidase; Avi, avicelase; CMC, CM-cellulase; Amy, amylase; Pec, pectinase; Xyl, xylanase; and Pro, proteinase.

1982. To test enzyme activity, *L. edodes* strains were pre-cultured on PDA and transferred to chromogenic media containing each of 0.5% carbon source as enzymatic substrate [CM-cellulose and avicel (Sigma, USA) for CM-cellulase and avicelase, respectively, D-cellobiose (Sigma, USA) for β -glucosidase, starch from potato (Sigma, USA) for amylase, polygalacturonic acid (MP Biomedical, France) for pectinase, skin milk (Sigma, USA) for protease, and xylan from oat spelts (Sigma, USA) for xylanase], 0.1% yeast nitrogen base (Difco, USA) as fundamental nitrogen source, 0.5% dyes (Congo Red, Sigma, USA) for chromogenic reaction, and 1.5% agar powder (Hyun *et al.*, 2006; Yoon *et al.*, 2007). After incubation at 25°C for 20 days, clear zone formed on chromogenic media by reaction between enzyme produced by the strains and chromogenic substrates was measured for activity assessment. The clear zone was measured from the inoculation point in the center of media plate to the end of clear zone. These tests were repeated three times. Based on the size of clear zone diameter (mm), the extent of enzyme activity was evaluated as follows; weak activity (W: 0~20 mm), moderate activity (M: 20~50 mm), and strong activity (S: 50~80 mm).

The results of extracellular enzyme activity test of Sanjo-108Ho and its twenty basidiospore strains were given at Table 1. Among the seven tested enzymes, the parental

Table 2. Extracellular enzyme activities shown by monokaryotic basidiospore strains (S101-01 to S101-20) and its parental strain Sanjo-101Ho

Strains	Enzymes						
	β -Glu	Avi	CMC	Amy	Pec	Xyl	Pro
Sanjo-101Ho	W	W	W	W	W	W	S
S101-01	M	W	W	W	W	W	M
S101-02	W	W	W	W	W	W	W
S101-03	S	W	W	W	W	W	M
S101-04	M	M	W	W	W	W	M
S101-05	W	W	W	W	W	W	M
S101-06	W	W	W	W	W	W	W
S101-07	W	M	W	W	M	W	M
S101-08	S	S	W	M	W	W	M
S101-09	W	W	W	W	S	W	W
S101-10	M	W	M	M	W	W	M
S101-11	M	S	M	W	W	W	M
S101-12	W	W	W	W	W	W	M
S101-13	W	W	W	W	W	W	M
S101-14	W	S	W	W	M	W	M
S101-15	W	W	W	W	W	W	M
S101-16	S	W	W	W	W	W	M
S101-17	M	W	W	W	W	W	M
S101-18	W	W	W	W	S	W	W
S101-19	W	W	M	W	W	W	W
S101-20	W	M	W	W	W	W	M

S, Strong activity; M, Moderate activity; W, Weak activity. β -Glu, β -glucosidase; Avi, avicelase; CMC, CM-cellulase; Amy, amylase; Pec, pectinase; Xyl, xylanase; and Pro, proteinase.

strain showed strong activity of β -glucosidase and amylase, and weak activity of CM-cellulase, avicelase, pectinase, protease, and xylanase. The twenty basidiospore strains showed different extent of extracellular enzyme activities compared to those of the parental strain. The extent of activity of the seven tested enzymes quite varied among the twenty basidiospore strains. The results indicate that the property of extracellular enzyme production contained in dikaryotic parental strain is not identically delivered to its progeny monokaryotic basidiospores. As shown at Table 2, the property of extracellular enzyme production is more diverse among the basidiospore strains.

Regarding enzyme type, strong β -glucosidase was popularly displayed in most of basidiospore strains at Table 1. No strong protease was detected in both the parental strain and all of its basidiospore strains. Despite that weak activity of avicelase, CM-cellulase, amylase, and xylanase was found in the parental Sanjo-108Ho, several its basidiospore strains showed strong activity of these enzymes. These data show that the parental Sanjo-108Ho contains good genetic sources for strong extracellular enzymes that degrade polymeric compounds such as starch, cellulose, and xylan. The basidiospore strain S108-01 showed strong activity of CM-cellulase, avicelase, pectinase, amylase, and xylanase. Among the twenty strains,

this strain was the best strain that has strong ability of producing broad range of extracellular enzymes. The finding of this S108-01 strain suggests that examination of basidiospore property will be very useful for the selection of progeny strains that have enhanced ability of extracellular enzyme production.

To gain further insight on the biochemical property shown by parental dikaryotic strain and its progeny monokaryotic basidiospore strain, we extended examination to another dikaryotic strain Sanjo-101Ho and its progeny twenty basidiospore strains (Table 2). This parental strain showed mostly weak activity in the tested enzymes except for protease. Most of basidiospore strains also showed weak activity against the tested enzymes. There were also variations in the extent of enzyme activity among the twenty basidiospore strains. Strong activity of β -glucosidase, CM-cellulase, avicelase, amylase, and pectinase was detected in some of basidiospore strains. No strong xylanase activity was shown both by the parental and basidiospore strains, suggesting that Sanjo-101Ho is not good source of xylanase with strong activity. The property of strong protease activity shown by Sanjo-101Ho was not reproduced in any basidiospore strains. As shown at Table 1 results, it is also demonstrated at Table 2 results that the ability of extracellular enzyme production shown by dikaryotic strain can be changed in its progeny basidiospore strains. When it comes to the comparison of the two shiitake strains, Sanjo-108Ho has much better ability of producing extracellular enzymes than Sanjo-101Ho. This superiority in enzymatic function was also clearly displayed in the basidiospore strains of Sanjo-108Ho.

Overall, the analysis of extracellular enzyme activity based on chromogenic media allowed us to compare and evaluate the ability of enzyme production in both the parental and progeny strains of two shiitake mushrooms. The production of extracellular enzymes by cultivated shiitake mushroom was also reported by Leatham (1985). Considering that information on the evaluation of diverse enzymes in shiitake strains and their basidiospore strains has been rarely available, the results of the present study are quite meaningful. The assessment of seven enzymes tested in this study was found to be quite valuable since the biochemical property of basidiospore strains could be evaluated by the extent of activity shown by them. Subsequently, we could differentiate basidiospore strains that are hard to differentiate at monokaryotic stage based on morphology and growth property on general mushroom media. For the breeding of noble hybrid strain, crossing of two different mating partners of monokaryotic basidiospore strains is common method (Lee *et al.*, 1993). For

this crossing work, the use of monokaryotic basidiospore strains with good traits for breeding purpose leads to increase the chance of developing noble strains with better traits and improved quality. Growth property on mushroom media and/or general fungal media such as PDA or MEA has been usually used for the selection of monokaryotic basidiospore strains. This method obviously has limit in the evaluation of monokaryotic basidiospore strains because most basidiospores grow well on those media. Thus, the assessment of extracellular enzyme activity using chromogenic plate method used in this study will get over this limitation and usefully be applied to the breeding process of *L. edodes*.

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